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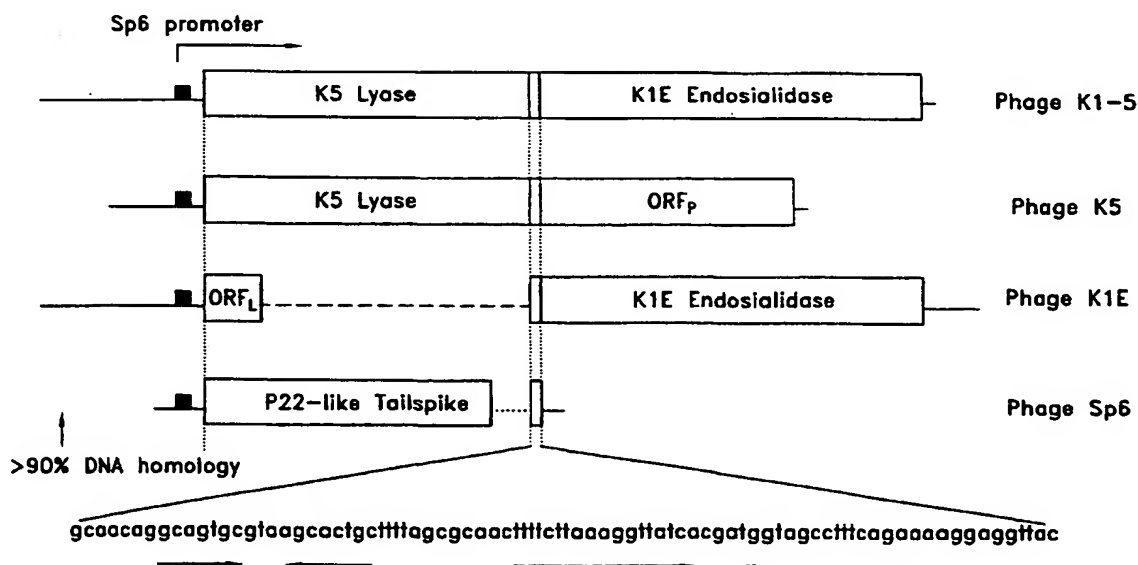(43) International Publication Date
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(54) Title: BACTERIOPHAGE HAVING MULTIPLE HOST RANGE

(57) Abstract: The present invention discloses compositions and methods for the prophylaxis and treatment of bacterial infections by the use of polyvalent bacteriophage having multiple host range.

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Bacteriophage Having Multiple Host Range

Field of the Invention

5   The present invention discloses compositions and methods for the prophylaxis and treatment of bacterial infections by the use of polyvalent bacteriophage having multiple host range.

Background of the Invention

Escherichia coli capsular polysaccharides (K antigens) have often been

10   associated with increased virulence (17). The K1 antigen in particular increases the invasiveness of *E. coli*, and these strains are often involved in cases of meningitis and septicemia (32). These polysaccharide coats also act as recognition sites for bacteriophages, which often carry tail spikes that contain polysaccharide depolymerization activities. Several K1 specific phages have been described (10), one

15   of which, ΦK1E, was found to possess N-acetylneuraminidase (endosialidase) as a part of the tail fiber protein (37). This enzyme catalyzes the cleavage of α-2,8-linked poly-N-acetylneuraminic acid carbohydrate polymer of the K1 capsule. It has been suggested that the tail fiber protein is involved in both adsorption to the cell surface and penetration into the cell by enzymatically degrading the polysaccharide capsule. The

20   ΦK1E endosialidase gene has been cloned and sequenced (20). A similar gene has been cloned and sequenced from ΦK1F (29).

ΦK5 is a related bacteriophage specific for *E. coli* strains that display the K5 antigen, a polymer consisting of a repeating structure of 4-linked a-N-acetylglucosamine and β-glucuronic acid (N-acetyl heparosin). In this case, ΦK5 encodes a tail associated

25   K5 specific lyase protein that is also responsible for attachment to the cell surface and degradation of the K5 polysaccharide capsule (12,14). Phage have also been found that are specific for other *E. coli* polysaccharide antigens including K3, K7, K12, K13, and K20 (26,27); all probably possess specific polysaccharide depolymerization activities as part of the phage particle.

Both ΦK5 and ΦK1E have a *Salmonella* phage SP6-like promoter upstream of their tail proteins as well as a region of sequence similarity, which is just downstream of the lyase gene of ΦK5 and just upstream of the endosialidase gene of ΦK1E (6). The sequences upstream of the tail gene promoters in ΦK1E, and ΦK5 are highly similar as well. ΦK5, ΦK1E and SP6 share a common morphology and life cycle, suggesting that they may be closely related.

Antibiotics superseded the potential use of bacteriophage in the treatment of infections. The extensive use of antibiotics has led to antibiotic- resistant bacterial pathogens. Thus, investigators have reassessed bacteriophage therapy and prophylaxis. However, one major obstacle that is frequently raised to the use of bacteriophage is that of their excessively narrow host range. There is a need for bacteriophage having multiple host range for use in therapy and prophylaxis.

Summary of the Invention

A virulent double stranded DNA bacteriophage, ΦK1-5 has been isolated and was found to be capable of infecting *E. coli* strains that possess either the K1 or the K5 polysaccharide capsule. Electron micrographs show that the virion consists of a small icosohedral head with short tail spikes, similar to members of the *Podoviridae* family. DNA sequence analysis of the region encoding the tail fiber protein showed two open reading frames encoding previously characterized hydrolytic phage tail fiber proteins. The first is the K5 lyase protein gene of ΦK5, which allows this phage to specifically infect K5 *E. coli* strains. A second open reading frame encodes a protein almost identical in amino acid sequence to the N-acetylneuraminidase (endosialidase) protein of ΦK1E, which allows this phage to specifically infect K1 strains of *E. coli*. We provide experimental evidence that mature phage particles contain both tail fiber proteins, and mutational analysis indicates that each protein can be independently inactivated. A comparison of the tail gene regions of ΦK5, ΦK1E, and ΦK1-5 shows that the genes are arranged in a modular or cassette configuration. The demonstration that a phage can contain multiple tail proteins that expand its host range is useful in generating phage with broad-spectrum antibacterial properties for therapy and prophylaxis of bacterial infections.

Brief Description of the Drawings

Figure 1.   Electron micrograph of ΦK1-5 negatively stained with phosphotungstic acid at a magnification of X115,500. Morphologically this phage and can be classified in the *Podoviridae* family which includes T7 and SP6.

Figure 2.   Comparison of the coding regions of the tail proteins of ΦK1-5, ΦK5, and ΦK1E. All three phages share sequence similarity in the upstream region (which contains an SP6 promoter) as well as an 85-base intergenic region. Just downstream of the promoter, ΦK1-5 and ΦK5 encode a lyase protein and ΦK1E encodes ORF_L. Immediately following the termination codons of the lyases or ORF_L is the intergenic region that contains a potential hairpin structure, the first of which could be a Rho-independent transcription terminator. Immediately following this, ΦK1-5 and ΦK1E encode an endosialidase where ΦK5 encodes ORF_P. None of the three phages have any coding regions downstream, and the DNA molecule ends in all three cases. No sequence similarity exists in this terminal region.

Figure 3.   Two possible models for the arrangement of the tail proteins on the phage capsid. (a) There are three copies of each tail forming a hexamer. (b) There are six copies of each tail. One is attached to the head and is part of the "core" of the tail. The other is then attached to the first tail protein, in effect making a longer tail fiber with two different enzymatic activities.

Figure 4.   Comparison of the coding regions of the tail proteins of ΦK1-5, ΦK5, ΦK1E, and SP6.

Brief Description of the Sequences

SEQ ID NO:1 is the DNA sequence of the tail gene region of ΦK1-5.

SEQ ID NO:2 is the DNA sequence of ΦSP6 tail gene.

SEQ ID NO:3 is the DNA sense sequence of ΦK1-5.

SEQ ID NO:4 is the DNA antisense sequence of ΦK1-5.

Brief Description of the Biological Deposits

ΦK1-5 was deposited as ATCC Accession No. PTA-3495 on July 2, 2001 with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, VA

5   20110-2209, USA. This deposit was made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures maintenance of a viable culture of the deposit for 30 years from date of deposit. The deposit will be made available by ATCC under the terms of the Budapest Treaty, and

10   subject to an agreement between Applicant and ATCC which assures permanent and unrestricted availability of the progeny of the culture of the deposit to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be

15   entitled thereto according to 35 USC § 122 and the Commissioner's rules pursuant thereto (including 37 CFR § 1.14). Availability of the deposited strain is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

20   Detailed Description of the Preferred Embodiment

We have discovered that it is possible to express more than one host specific tail protein in a single bacterial viral strain and that expression of these tail proteins permits the virus to infect multiple specific hosts. This discovery facilitates the genetic engineering of phage with expanded host ranges. For example, there are phage that can

25   infect *E. coli* strains that contain the K1 polysaccharide in their outer capsule. Such *E. coli* bacterial strains are often involved in meningitis and septicemia, as K1 polysaccharide increases the invasiveness of the bacteria (32). A phage (ΦK1E) possesses a tail protein with endosialdase activity that can infect strains of *E. coli* containing the K1 polysaccharide. This endosialdase allows the phage ΦK1E to

specifically attach and degrade the K1 polysaccharide (37). Similarly, phage ΦK5 can infect K5 strains of *E. coli*. The K5 strains of *E. coli* are commonly a cause of urinary tract infections (11). The phage ΦK5 contains a tail protein that has lyase activity that allows this phage to attack the bacterial K5 capsule (12, 14). We have demonstrated

5   that it is possible for a phage to have both tail proteins, the K1 endosialdase as well as the K5 lyase. Such a phage, which we have designated phage ΦK1-5, has an expanded host range as it can infect both *E. coli* K1 and *E. coli* K5. We have demonstrated that this expanded host range capability is due to the capacity of this ΦK1-5 phage to display both tail proteins, a K1 endosialidase as well as a K5 lyase. We have demonstrated

10   through sequence analysis of ΦK1-5 phage tail protein genes that they are arranged in a modular or cassette structure, indicating that the host range of phages can be broadened for other K antigens, and even other bacteria species by recombinational techniques. The demonstration that a phage can contain multiple tail proteins that expand its host range is envisioned as being useful in generating phage with broad-spectrum

15   antibacterial properties for the therapy and prophylaxis of infectious diseases. Recently there has been a renewed interest in the use of phages to treat and prevent bacterial infections (for a review see Barrow and Soothill, 1997, *Trends Microbiol.* 5(7), 268-271). ΦK1-5 is highly lytic, non-lysogenic, very stable, and kills bacteria rapidly, all features that make it a good candidate for phage therapy. The phage ΦK1-5 has an

20   additional advantage because it recognizes and attaches to the same structure(s) that confer virulence to the bacteria. In addition, bacteria that become resistant to phage usually have lost the polysaccharide capsule and are no longer virulent. Given these findings, ΦK1-5 is envisioned as being used as a general platform phage for therapeutic and prophylatic applications in which host specificity will be altered by engineering the

25   tail protein genes. The capacity to engineer the expression of tail proteins is also envisioned as providing for phage that can transfer genes to organisms that are not normally infected by phage. Such a goal is to be achieved by expressing a mammalian viral protein on the tail of the phage to enable such a phage to transfer its genetic material into a mammalian cell. Phage with this capability will be of use in gene

30   therapy applications.

Referring to Figure 1, ΦK1-5 is an isolated bacteriophage consisting of an icosohedral head with a small tuft of short tail fibers that is able to infect and replicate on either K1 or K5 strains of *E. coli*. It appears that its ability to replicate on these strains is due to the fact that it encodes two different hydrolytic tail fiber proteins. One

5   is an endosialidase protein, almost identical to a similar protein from ΦK1E, that allows it to attach to and degrade the K1 polysaccharide capsule. The other is almost identical to a lyase protein that has been shown to allow ΦK5 to attach to and degrade the K5 polysaccharide capsule. This is the first example of a phage that has a dual host specificity based on having two different tail proteins.

10   Referring to Figure 2, all three of these phages share sequence similarity upstream of the region encoding the tail proteins and all have an ΦSP6-like promoter that probably drives transcription of the tail gene(s). In ΦK1-5 and ΦK5, the first gene downstream of this promoter is the K5 lyase protein. ΦK1E does not encode this protein and instead has a 111 amino acid ORF (ORF$_L$) of unknown function. Immediately

15   downstream of the K5 lyase proteins of ΦK1-5 and ΦK5, and downstream of ORF$_L$ in ΦK1E is an 85 base region of similarity between all three phages. This region contains two strong symmetrical elements that may be involved in transcription termination. Further downstream, phages ΦK1-5 and ΦK1E encode the endosialidase gene. ΦK5 does not encode this gene but instead encodes the 523 amino acid ORF$_P$.

20   ΦK1-5 is a bacteriophage that we isolated from sewage using a K5 strain of *E. coli* as a host. By analyzing the host range of ΦK1-5, we found that it can replicate on either K1 or K5 strains. DNA sequence analysis of the tail fiber genes revealed that it encodes both a K5 lyase protein similar to that of ΦK5 and an endosialidase protein similar to that of ΦK1E. The arrangement of these genes indicates that phage host range

25   can be broadened or changed by the acquisition of new tail genes by recombination in nature or by technology in the laboratory.

ΦK5 also is able to replicate on K95 strains of *E. coli* (28). Since ORF$_P$ is in a position analogous to that of the endosialidase of ΦK1-5, it is also envisioned as a tail protein responsible for growth on K95 strains. Another K antigen specific phage,

30   ΦK20, is also able to lyse two different types of *E. coli* hosts, those that possess the K5 antigen and those that possess the K20 polysaccharide (26). We envision ΦK20 as

carrying a K5 lyase protein similar to the ΦK5/ΦK1-5 protein along with a K20 specific hydrolytic tail protein. Phages have also been isolated that are specific to the capsular antigens K3, K7, K12, and K13 of *E. coli*. Presumably these phages have corresponding K specific hydrolytic tail proteins. We envision other phages having

5   multiple specificities with other combinations of K antigens.

The host range of a bacteriophage is expanded beyond *E. coli* by expressing the genes encoding multiple different host tail proteins, a constraint being an understanding of the mechanism by which the tail protein is attached to the capsid structure of a phage. The N-terminus of the T7 tail protein is thought to be involved in attachment (29).

10 Neither the endosialidase nor the K5 lyase has this region, or any other region similar to any other tail protein (or with each other). Morphologically, ΦK1E, ΦK5 and ΦK1-5, are similar to *Salmonella* phage P22. The tail protein of P22 has been extensively studied and is also a hydrolytic protein involved in degradation of the *Salmonella* typhimurium O antigen. This protein is a homotrimer with six copies per phage (30).

15 The gp17 tail-fiber of T7 is also a trimer with 6 copies of the trimer per phage particle (33). The endosialidase of ΦK1E is also a trimer (20), but it has yet to be shown that there are 6 copies of the trimer per phage particle. Bacteriophage 63D is another newly characterized sialidase containing phage in which it has been shown by electron microscopy that the sialidase is present with 6 copies per particle (21). This phage is

20 quite different morphologically from ΦK1E, ΦK5, and ΦK1-5 and has a long tail similar to that of bacteriophage lambda, with the sialidase located at the end of the tail. Six copies of a trimeric tail protein appear to be a general structural motif. Assuming that the endosialidase and K5 lyase are also arranged in six copies per virion, it is interesting to speculate how the two tail proteins are arranged on the head structure of

25 ΦK1E. They may be arranged in an alternating fashion where there are three copies of each (Figure 3a). In the case of P22, there is evidence that only three copies of the tail are needed for infection (16) suggesting that this model is theoretically possible. The fact that there are no common sequence similarities between the two tail proteins argues against this model, since one might predict a common motif within the tail proteins that

30 is required to attach to similar regions of the head structure. An alternative model is that there may be 6 copies of each tail protein, one attached to the other (Figure 3b). Since

the N-terminus of the T7 tail protein is thought to be involved in attachment of the tail protein to the head structure, this region of the protein and similar regions of other tail proteins (or alternative regions of tail proteins that mediate attachment of one to the other) are envisioned as serving the attachment function, so an understanding of the mechanism no longer acts as a constraint.

Our findings indicate that phages ΦK5, ΦK1E, and ΦK1-5 all share a region of sequence similarity upstream of the tail proteins. This region contains a *Salmonella* phage SP6 promoter. The DNA sequence surrounding the promoter described in Nam et al., Gene 1986, 46:57 matched the analogous sequence in ΦK1-5. Based on this information, we designed a primer from this region to sequence downstream of the analogous region in phage ΦSP6. DNA sequencing identified an open reading frame that has a high degree of amino acid similarity to phage P22 tail protein. ΦP22 is a well characterized *Salmonella* phage that has a similar morphology to ΦSP6, but has a very different life cycle. (ΦP22 is lysogenic like *E. coli* phage lambda, and ΦSP6 is lytic like T7 or the three K antigen phages). The P22 tail protein also has a polysaccharide degradation activity. Immediately downstream of the SP6 tail gene lies the 85 base pair intergenic region common to ΦK5, ΦK1E and ΦK1-5, and shortly after that the DNA molecule ends. Figure 4 compares the regions encoding the tail proteins in all four phage. The SP6 tail protein is in the analogous position as the lyase protein of ΦK1-5 and ΦK5 and ORF$_L$ of ΦK1E. ΦSP6 shares the cassette structure of the three K phages, indicating that all four are closely related and differ mainly in the tail proteins. We envision replacing the lyase protein of ΦK1-5 with the SP6 tail to create a phage that can attack *Salmonella* and K1 *E. coli*. It should be easy to construct by homologous recombination because of the common sequence upstream of the tail proteins and the common 85 base sequence between the two tail proteins. An alternative is to create a phage that can attack *Salmonella*, K1 *E. coli* and K5 *E. coli* by designing a construct to encode all three proteins. In the case of ΦK1-5, we have evidence that broad host range evolved by the acquisition of a second specific tail protein. Thus, under the theory of modular evolution, in which duplications and rearrangements of regions within tail fiber genes of different phages seem to mediate changes in host specificity, we envision

increasing host range even further by designing the acquisition of a third specific tail protein, a fourth specific tail protein, and multiple specific tail proteins.

Definitions

5   The term "isolated" requires that a material be removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally occurring phage present in a natural system is not isolated, but the same phage, separated from some or all of the coexisting materials in the natural system, is isolated.

The term "purified" does not require absolute purity; rather it is intended as a 10   relative definition, with reference to the purity of the material in its natural state. Purification of natural material to at least one order of magnitude, preferably two or three magnitudes, and more preferably four or five orders of magnitude is expressly contemplated.

The term "enriched" means that the concentration of the material is at least about 15   2, 5, 10, 100, or 1000 times its natural concentration (for example), advantageously 0.01% by weight. Enriched preparations of about 0.5%, 1%, 5%, 10%, and 20% by weight are also contemplated.

Multiple Host Specificity Based On Multiple Different Host Tail Proteins

20   Phage therapy capitalizes on the ability of phage to lyse bacteria. With the increasing incidence of antibiotic resistant bacteria, there is a need to counteract them. The present invention meets that need by overcoming the additional disadvantage frequently raised to the use of phage, which is their excessively narrow host range.

A prototype bacteriophage has a head and a tail. The head is an icosahedron. 25   The tail consists of a hollow core. The whole apparatus functions as a syringe for injection of phage DNA into the interior of a bacterial cell. The life cycle is that viral genes are expressed, virions are assembled, and cellular lysis releases infectious particles into the medium. In this way, the bacteriophage kill the host pathogen.

One way of evading a host's antibody response is for a bacterium to coat itself 30   with a capsule. A capsule is a network of polymers that is usually composed of polysaccharides, but may be composed of proteins or protein-carbohydrate mixtures,

and that resembles host tissue polymers. In *E. coli*, over 70 different polysaccharide or protein K antigens are currently recognized by the World Health Organization.

Bacteriophage carry a tail protein that attaches to a surface structure of a bacterium by which the viral genome enters the infected cell. Some bacteriophage

5   possess a tail protein that contains capsule-degrading enzymatic activity. These enzymes facilitate penetration by bacteriophage of the bacterial cell capsule. In K1 specific bacteriophage, the tail protein is a neuranimidase. In K5 specific bacteriophage, the tail protein is a lyase. In other K specific bacteriophage, the tail protein is another hydrolytic protein.

10   Phylogenetic classification indicates that gram-negative bacteria form one group of bacteria. *Escherichia, Shigella, Enterobacter* and *Salmonella* are genera of bacteria that are closely related to each other. *Yersinia* and *Vibrio* are the genera next most closely related to the *E. coli* group. *Serratia* and *Klebsiella* are the genera next most closely related to the *E. coli* group. *Campylobacter* is also the genera of Proteobacteria

15   phylum. The genera *Legionella, Pseudomonas*, and *Neisseria* are more distantly related. *Bordetella*'s relationship is unknown. *Helicobacter* is the most distantly related genus in the *E. coli* group of gram-negative bacteria. The gram-positive bacteria form another group, with *Listeria* more closely related to the gram-positive cocci *Staphylococcus* and *Streptococcus, Enterococcus* and *Clostridium*, than to other gram-positive rods.

20   *Corynebacterium* is most closely related to *Mycobacterium*. The spirochetes *Treponema* and *Borrelia* form a third phylogenetic group, while *Chlamydia* is related more distantly to this group. Despite the genetic diversity represented by pathogenic bacteria, similar strategies for overcoming host defenses have evolved in very different types of bacteria. We contemplate using our invention against but not limited to the

25   following pathogenic bacteria presented in the table below.

Pathogen	Disease	Phage
Escherichia	Hemmorrhagic colitis; thrombocytopenia; hemolytic uremic syndrome	+, ATCC
Shigella	Dysenteria	+, ATCC
Salmonella	Typhus	+, ATCC
Enterobacter	Urinary tract infections	+, ATCC
Yersinia	Plague	+, ATCC
Vibrio	Cholera; severe diarrhea, rapid dehydration	+, ATCC
Legionella	Legionnaires' disease: malaise, myalgia, fever, headache, respiratory illness	−
Pseudomonas	Opportunistic infections	+, ATCC
Neisseria	Bacterial meningitis	+, ATCC
Bordetella	Pertussis (whooping cough)	+, ref[a]
Helicobacter	Gastritis, peptic ulcers, possibly stomach cancer	+, ref[b]
Listeria	Listeriosis (meningitis)	+, ATCC
Staphylococcus	Abscesses, pneumonia, endocarditis, toxic shock	+, ATCC
Streptococcus	Scarlet fever, rheumatic fever, toxic shock	+, ATCC
Enterococcus	Urinary tract infections	+, ATCC
Clostridium	Tetanus	+, ATCC
Corynebacterium	Diphtheria	+, ATCC
Mycobacterium	Tuberculosis: cough, weight loss, lung lesions; infection can spread to other organ systems	+, ATCC
Treponema	Syphilis	+, ref[c]
Borrelia	Lyme disease: rash, fever, neurological and cardiac abnormalities, arthritis	+, ref[d]
Campylobacter	Campylobacter enteritis: abdominal pain, diarrhea, fever	+, ATCC
Chlamydia	Trachoma, genital infections, conjunctivitis, infant pneumonia	+, ref[e]
Haemophilus	Brazilian purpuric fever: purulent conjunctivitis, fever, vomiting	+, ATCC
Serratia	Opportunistic infection in neonates	+, ATCC
Klebsiella	pneumonia	+, ATCC

Where: "+, ATCC" indicates the presence of a corresponding bacteriophage(s) in the ATCC; "+, ref" indicates that information on the excisting corresponding bacteriophage can be found in the following scientific literature: ref[a] - Holzmayer TA, *et al.* 1988 *Zentralbl Bakteriol Mikrobiol Hyg* [A] 269(2):147-55; Gol'tsmaier TA, *et al.* 1987 *Zh Mikrobiol Epidemiol Immunobiol* 5:9-13; ref[b] - Heintschel von Heinegg E, *ey al.* 1993 *J Med Microbiol* 38(4):245-9; ref[c] - Ritchie AE, *et al.* 1978 *Vet Rec* 103(2):34-5; ref[d] - Eggers CH, *et al.* 2000 *J Mol Microbiol Biotechnol*2(4):365-73; ref[e] - Hsia R, *et al.* 2000 *Microbes Infect* 2(7):761-72; Hsia RC, *et al.* 2000 *Microbiology* 146 (Pt 7):1651-60.

In an embodiment of the present invention, a phage has a dual host specificity based on having two different host tail proteins. In another embodiment, a phage has a triple host specificity based on having three different host tail proteins. In a further embodiment, a phage has a quadruple host specificity based on having four different

5   host tail proteins. And so forth, so that in an additional embodiment, a phage has a multiple host specificity based on having multiple different host tail proteins.

In another embodiment of the present invention, a phage having a hydrolytic tail protein has a dual host specificity based on having two different hydrolytic tail proteins. In another embodiment, a phage having a hydrolytic tail protein has a triple host

10   specificity based on having three different hydrolytic tail proteins. In a further embodiment, a phage having a hydrolytic tail protein has a quadruple host specificity based on having four different hydrolytic tail proteins. And so forth, so that in an additional embodiment, a phage having a hydrolytic tail protein has a multiple host specificity based on having multiple different hydrolytic tail proteins.

15   In another embodiment of the present invention, a phage having a K specific hydrolytic tail protein has a dual host specificity based on having two different K specific hydrolytic tail proteins. In another embodiment, a phage having a K specific hydrolytic tail protein has a triple host specificity based on having three different K specific hydrolytic tail proteins. In a further embodiment, a phage having a K specific

20   hydrolytic tail protein has a quadruple host specificity based on having four different K specific hydrolytic tail proteins. And so forth, so that in an additional embodiment, a phage having a K specific hydrolytic tail protein has a multiple host specificity based on having multiple different K specific hydrolytic tail proteins.

A first example of a phage that has a dual host specificity is ΦK1-5. An

25   example of a phage having a triple host specificity is ΦK1-5 having a third different tail protein. An example of a phage having a quadruple host specificity is ΦK1-5 having a third and fourth different tail protein. And so forth, so that an example of a phage having a multiple host specificity is ΦK1-5 having multiple different host tail proteins.

A second example of a phage that has a dual host specificity is ΦK1E having a

30   second different tail protein, like K1-5. An example of a phage having a triple host specificity is ΦK1E having a second and third different tail protein. An example of a

phage having a quadruple host specificity is ΦK1E having a second, third and fourth different tail protein. And so forth, so that an example of a phage having a multiple host specificity is ΦK1E having multiple different host tail proteins.

A third example of a phage that has a dual host specificity is ΦK5 having a second different tail protein, like K1-5. An example of a phage having a triple host specificity is ΦK5 having a second and third different tail protein. An example of a phage having a quadruple host specificity is ΦK5 having a second, third and fourth different tail protein. And so forth, so that an example of a phage having a multiple host specificity is ΦK5 having multiple different host tail proteins.

Another example of a phage that has a multiple host specificity based on having different host tail proteins is a phage that infects *Escherichia* and additionally infects a bacterium selected from the group consisting *of Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Enterococcus, Clostridium, Corynebacterium, Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia* and *Klebsiella*.

Another example of a phage that has a multiple host specificity based on having different host tail proteins is a phage that infects *Shigella* and additionally infects a bacterium selected from the group consisting of *Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Enterococcus, Clostridium, Corynebacterium, Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia* and *Klebsiella*.

Another example of a phage that has a multiple host specificity based on having different host tail proteins is a phage that infects *Salmonella* and additionally infects a bacterium selected from the group consisting *of Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Enterococcus, Clostridium, Corynebacterium, Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia* and *Klebsiella*.

Another example of a phage that has a multiple host specificity based on having different host tail proteins is a phage that infects *Enterobacter* and additionally infects a bacterium selected from the group consisting *of Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella,*
5   *Helicobacter, Listeria, Staphylococcus, Streptococcus, Enterococcus, Clostridium, Corynebacterium, Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia* and *Klebsiella.*

Another example of a phage that has a multiple host specificity based on having different host tail proteins is a phage that infects *Yersinia* and additionally infects a
10   bacterium selected from the group consisting of *Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Enterococcus, Clostridium, Corynebacterium, Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia* and *Klebsiella.*

15   Another example of a phage that has a multiple host specificity based on having different host tail proteins is a phage that infects *Vibrio* and additionally infects a bacterium selected from the group consisting *of Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Enterococcus, Clostridium,*
20   *Corynebacterium, Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia* and *Klebsiella.*

Another example of a phage that has a multiple host specificity based on having different host tail proteins is a phage that infects *Legionella* and additionally infects a bacterium selected from the group consisting of *Escherichia, Shigella, Salmonella,*
25   *Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Enterococcus, Clostridium, Corynebacterium, Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia* and *Klebsiella.*

Another example of a phage that has a multiple host specificity based on having
30   different host tail proteins is a phage that infects *Pseudomonas* and additionally infects a bacterium selected from the group consisting of *Escherichia, Shigella, Salmonella,*

Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Enterococcus, Clostridium, Corynebacterium, Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia and *Klebsiella*.

Another example of a phage that has a multiple host specificity based on having different host tail proteins is a phage that infects *Neisseria* and additionally infects a bacterium selected from the group consisting of *Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Enterococcus, Clostridium, Corynebacterium, Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia* and *Klebsiella*.

Another example of a phage that has a multiple host specificity based on having different host tail proteins is a phage that infects *Bordetella* and additionally infects a bacterium selected from the group consisting of *Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Enterococcus, Clostridium, Corynebacterium, Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia* and *Klebsiella*.

Another example of a phage that has a multiple host specificity based on having different host tail proteins is a phage that infects *Helicobacter* and additionally infects a bacterium selected from the group consisting of *Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Enterococcus, Clostridium, Corynebacterium, Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia* and *Klebsiella*.

Another example of a phage that has a multiple host specificity based on having different host tail proteins is a phage that infects *Listeria* and additionally infects a bacterium selected from the group consisting of *Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Enterococcus, Clostridium,*

Corynebacterium, Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia and *Klebsiella*.

Another example of a phage that has a multiple host specificity based on having different host tail proteins is a phage that infects *Staphylococcus* and additionally infects a bacterium selected from the group consisting of *Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Enterococcus, Clostridium, Corynebacterium, Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia* and *Klebsiella*.

Another example of a phage that has a multiple host specificity based on having different host tail proteins is a phage that infects *Streptococcus* and additionally infects a bacterium selected from the group consisting *of Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Enterococcus, Clostridium, Corynebacterium, Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia* and *Klebsiella*.

Another example of a phage that has a multiple host specificity based on having different host tail proteins is a phage that infects *Enterococcus* and additionally infects a bacterium selected from the group consisting *of Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Enterococcus, Clostridium, Corynebacterium, Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia* and *Klebsiella*.

Another example of a phage that has a multiple host specificity based on having different host tail proteins is a phage that infects *Clostridium* and additionally infects a bacterium selected from the group consisting of *Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Clostridium, Corynebacterium, Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia* and *Klebsiella*.

Another example of a phage that has a multiple host specificity based on having different host tail proteins is a phage that infects *Corynebacterium* and additionally infects a bacterium selected from the group consisting of *Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria,*

5   *Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Clostridium, Corynebacterium, Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia* and *Klebsiella.*

Another example of a phage that has a multiple host specificity based on having different host tail proteins is a phage that infects *Mycobacterium* and additionally infects

10   a bacterium selected from the group consisting of *Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Clostridium, Corynebacterium, Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia* and *Klebsiella.*

15   Another example of a phage that has a multiple host specificity based on having different host tail proteins is a phage that infects *Treponema* and additionally infects a bacterium selected from the group consisting of *Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Clostridium, Corynebacterium,*

20   *Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia* and *Klebsiella.*

Another example of a phage that has a multiple host specificity based on having different host tail proteins is a phage that infects *Borrelia* and additionally infects a bacterium selected from the group consisting of *Escherichia, Shigella, Salmonella,*

25   *Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Clostridium, Corynebacterium, Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia* and *Klebsiella.*

Another example of a phage that has a multiple host specificity based on having

30   different host tail proteins is a phage that infects *Campylobacter* and additionally infects a bacterium selected from the group consisting of *Escherichia, Shigella, Salmonella,*

Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Clostridium, Corynebacterium, Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia and *Klebsiella.*

5   Another example of a phage that has a multiple host specificity based on having different host tail proteins is a phage that infects *Chlamydia* and additionally infects a bacterium selected from the group consisting of *Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Clostridium, Corynebacterium,*

10   *Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia* and *Klebsiella.*

Another example of a phage that has a multiple host specificity based on having different host tail proteins is a phage that infects *Haemophilus* and additionally infects a bacterium selected from the group consisting of *Escherichia, Shigella, Salmonella,*

15   *Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Clostridium, Corynebacterium, Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia* and *Klebsiella.*

Another example of a phage that has a multiple host specificity based on having

20   different host tail proteins is a phage that infects *Serratia* and additionally infects a bacterium selected from the group consisting of *Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Clostridium, Corynebacterium, Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus,*

25   *Serratia* and *Klebsiella.*

Another example of a phage that has a multiple host specificity based on having different host tail proteins is a phage that infects *Klebsiella* and additionally infects a bacterium selected from the group consisting of *Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella,*

30   *Helicobacter, Listeria, Staphylococcus, Streptococcus, Clostridium, Corynebacterium,*

Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia and *Klebsiella.*

Host Range Of Phage To Include Bacterial Cells Requiring Co-factors

5   In another embodiment of the present invention, a phage that has a multiple host specificity based on having multiple different host tail proteins additionally has a gene encoding a co-factor that permits it to grow in other types of bacteria. Having the proper tail proteins is necessary for a phage to infect a strain of bacteria, as illustrated by the present experiments. For example, a phage having different host tail proteins is

10   capable of infecting multiple hosts where the host expresses at least one of these host tail proteins. Nevertheless, additional factors may be necessary for a phage to infect other strains of bacteria. The present invention provides these phage where co- factors may also be needed to increase their host range.

         For example the *E. coli* phage lambda does not generally infect *Salmonella. E.*

15   *coli* phage lambda requires a functional *E. coli* Nus A gene for lambda promoted transcription/anti-termination of DNA$\rightarrow$ RNA to permit the virus to replicate and function. As the bacteria *Salmonella* does not have the *E. coli* Nus A gene, lambda cannot grow in *Salmonella*. When the *E. coli* Nus A gene is cloned into the lambda genome, this virus can then infect certain *Salmonella* stains. In a confirmatory

20   experiment, the *E. coli* genome was cut into fragments with restriction enzymes and the fragments cloned into a lambda library. When these lambda phage were plated on *Salmonella,* only those containing *E. coli* Nus A gene grew. Thus, lambda carrying the *E. coli* Nus A gene provides one example of how the host range of a phage can be expanded.

25   In another example of a phage that does not generally infect another type of bacteria, the gene that permits the virus to replicate and function in the bacteria may be known. If known, the gene can be cloned into the viral genome so that this virus can then infect other types of bacteria. If unknown, the gene can be identified, for example, by cutting the bacterial genome into fragments with restriction enzymes and then

30   cloning the fragments into a library of that virus. When these phage are plated on the bacteria, only those containing the gene will grow. Thus, the gene that permits the virus

to replicate and function in the bacteria can be identified. Once the gene is known, virus can be engineered to carry the gene. Thus, the host range of the phage can be expanded even when co-factors are necessary to grow in other types of bacteria.

Host Range of Phage To Include Mammalian Cells

5   In another embodiment of the present invention, a phage has mammalian host specificity based on incorporating the gene for a cell surface-receptor ligand into the phage genome such that it is expressed on the tail of the phage, thus faciliating receptor mediated endocytosis. Poul et al., J Mol Biol 1999, 288:203 and Larocca et al., FASEB

10   J 1999, 13:727 describe gene delivery to mammalian cells by bacteriophage expressing cell surface-receptor ligands as genetic fusions with phage coat proteins or presenting cell surface-receptor ligands on the coats of phage, with the goal being the development of gene therapy vectors. The present invention envisions substituting phage tail proteins.

15   A cell surface-receptor ligand is genetically fused to a phage tail protein or otherwise presented on the tails of phage. Nucleotide sequences encoding ligand-phage fusions or cell surface-receptor ligands themselves may also be modified by insertion of mammalian reporter genes to test for binding, internalization, and expression. Ultimately, the mammalian reporter gene is replaced by a therapeutic nucleotide

20   sequence.

The therapeutic nucleotide sequence encodes a factor having a therapeutic effect. In alternative embodiments, a factor is a protein cytocide, an antisense, a ribozyme, a dominant negative mutant, or a therapeutic protein (e.g., a growth factor, a hormone, a cytokine, a receptor, or a ligand). An example of receptor-mediated gene delivery using

25   bacteriophage vectors displaying ligands as genetic fusions with phage coat proteins or presenting cell surface-receptor ligands on the coats of phage is set forth in USP 6,054,312 to Larocca et al.

Methods of Making

30   In a different embodiment, the acquisition of new tail genes occurs by recombination in nature. In an alternative embodiment, the acquisition of new tail

genes is generated·by technology in the laboratory. Thus, the phage may be developed by selection or engineering.

Phage that have multiple specificities may be selected by assays to determine the host range of phages, such as plaque assays.

5   Alternatively, phage that have multiple specificities may be engineered by cloning the gene for a tail protein into a plasmid vector system and then incorporating this configuration into the phage of interest by an in vivo generalized recombination system in the host bacteria for the phage of interest; or by cloning the gene for a first tail protein into a plasmid vector system, and then cloning the gene for a second tail protein

10   into this carrying vector by in-frame fusion at the 3'- or 5'- end of the gene for the first tail protein, and then incorporating this configuration into the phage of interest by an in vivo generalized recombination system in the host bacteria for the phage of interest; or by cloning the gene for a first tail protein into a plasmid vector system, and then cloning the gene for a second tail protein into this carrying vector by in-frame fusion at the 3'-

15   or 5'- end of the gene for the first tail protein, and then cloning the gene for a third tail protein into this carrying vector by in-frame fusion at the 3'- or 5'- end of the genes for the first and second tail proteins, and so forth, and then incorporating this configuration into the phage of interest by an in vivo generalized recombination system in the host bacteria for the phage of interest.

20

<u>Methods of Using, Formulations, and Administration</u>

The present invention can be applied across the spectrum of bacterial diseases, by selecting or engineering phages, so that phages are developed that are specific for more than one bacterial strain of interest. In that way, a full array of polyvalent

25   bacteriophages is developed for virtually all the bacterial pathogens for man, his pets, livestock and zoo animals (whether mammal, avian, or pisciculture). Phage therapy will then be available:

1) as an adjunct to or as a replacement for those antibiotics and/or chemotherapeutic drugs that are no longer functioning in a bacteriostatic or bactericidal

30   manner due to the development of multi-drug resistance;

2) as a treatment for those patients who are allergic to the antibiotics and/or chemotherapeutic drugs that would otherwise be indicated; and

3) as a treatment that has fewer side effects than many of the antibiotics and/or chemotherapeutic drugs that would otherwise be indicated for a given infection.

Another embodiment of the present invention is the development of methods to treat bacterial infections in animals through phage therapy with the polyvalent bacteriophages described above. Hundreds of bacteriophages and the bacterial species they infect are known in the art. The present invention can be utilized to develop polyvalent bacteriophages that can be used to treat any and all infections caused by their host bacteria.

While it is contemplated that the present invention can be used to treat any bacterial infection in an animal and human, it is particularly contemplated that the methods described herein will be very useful as a therapy (adjunctive or stand-alone) in infections caused by drug-resistant bacteria. Experts report (See e.g. Gibbons, A., "Exploring New Strategies to Fight Drug-Resistant Microbes, Science, 21 Aug. 1993, pp. 1036-38) that at the present time, the drug-resistant bacterial species and strains listed below represent the greatest threat to mankind:

1. All of the clinically important members of the family *Enterobacteriaceae*, most notably but not limited to the following:

a) All the clinically important strains of *Escherichia*, most notably *E. coli*. One among a number of candidate wild-type phages against these particular pathogens that could be used as a starting point for the genetic engineering of the present invention would be θK1-5 having ATCC Accession No. # PTA-3495. (Note: For purposes of brevity, in all the following examples of pathogens, the corresponding wild-type phage will be indicated by the following phraseology: "Example of corresponding phage: ___".)

b) All the clinically important strains of *Klebsiella*, most notably *K. pneumoniae* (Example of corresponding phage: ATCC phage #23356-B1).

c) All the clinically important strains of *Shigella*, most notably *S. dysenteriae* (Example of corresponding phage: ATCC phage #11456a-B1).

d) All the clinically important strains of *Salmonella*, including *S. abortus-equi* (Example of corresponding phage: ATCC phage #9842-B1), *S. typhi* (Example of corresponding phage: ATCC phage #19937-B1) *S. typhimurium* (Example of corresponding phage: ATCC phage #19585-B1), *S. newport* (Example of corresponding

5   phage: ATCC phage #27869-B1), *S. paratyphi-A* (Example of corresponding phage: ATCC phage #12176-B1), *S. paratyphi-B* (Example of corresponding phage: ATCC phage #19940-B1), *S. potsdam* (Example of corresponding phage: ATCC phage #25957-B2), and *S. pollurum* (Example of corresponding phage: ATCC phage #19945-B1).

10   e) All the clinically important strains of *Serratia*, most notably *S. marcescens* (Example of corresponding phage: ATCC phage #14764-B1).

f) All the clinically important strains of *Yersinia*, most notably *Y. pestis* (Example of corresponding phage: ATCC phage #11953-B1).

g) All the clinically important strains of *Enterobacter*, most notably *E. cloacae*
15   (Example of corresponding phage: ATCC phage #23355-B1).

2. All the clinically important *Enterococci*, most notably *E. faecalis* (Example of corresponding phage: ATCC phage #19948-B1) and *E. faecium* (Example of corresponding phage: ATCC phage #19953-B1).

3. All the clinically important *Haemophilus* strains, most notably *H. influenzae*
20   (a corresponding phage is not available from ATCC for this pathogen, but several can be obtained from WHO or other labs that make them available publicly).

4. All the clinically important *Mycobacteria*, most notably *M. tuberculosis* (Example of corresponding phage: ATCC phage #25618-B1), *M. avium-intracellulare*, *M. bovis*, and *M. leprae*. (Corresponding phages for these pathogens are available
25   commercially from WHO, via The National Institute of Public Healthy & Environmental Protection, Bilthoven, The Netherlands).

5. *Neisseria gonorrhoeae* and *N. meningitidis* (Corresponding phage for both can be obtained publicly from WHO or other sources).

6. All the clinically important *Pseudomonads*, most notably *P. aeuruginosa*
30   (Example of corresponding phage: ATCC phage #14203-B1).

7. All the clinically important *Staphylococci*, most notably *S. aureus* (Example of corresponding phage: ATCC phage #27690-B1) and *S. epidermidis* (Corresponding phage are available publicly through the WHO, via the Colindale Institute in London).

8. All the clinically important *Streptococci*, most notably *S. pneumoniae* (Corresponding phage can be obtained publicly from WHO or other sources).

9. *Vibrio cholera* (Example of corresponding phage: ATCC phage #14100-B1).

There are additional bacterial pathogens too numerous to mention that, while not currently in the state of antibiotic-resistance crisis, nevertheless make excellent candidates for treatment with polyvalent bacteriophages in accordance with the present invention. Thus, all bacterial infections caused by bacteria for which there is a corresponding phage can be treated using the present invention.

Any phage strain capable of doing direct or indirect harm to a bacteria (or other pathogen) is contemplated as useful in the present invention. Thus, phages that are lytic, phages that are lysogenic but can later become lytic, and nonlytic phages that can deliver a product that will be harmful to the bacteria are all useful in the present invention.

The animals to be treated by the methods of the present invention include but are not limited to man, his domestic pets, livestock, pisciculture, and the animals in zoos and aquatic parks.

The polyvalent bacteriophages of the present invention can be used as a stand-alone therapy or as an adjunctive therapy for the treatment of bacterial infections. Numerous antimicrobial agents (including antibiotics and chemotherapeutic agents) are known in the art, which would be useful in combination with polyvalent bacteriophages for treating bacterial infections. Examples of suitable antimicrobial agents and the bacterial infections that can be treated with the specified antimicrobial agents are listed below. However, the present invention is not limited to the antimicrobial agents listed below as one skilled in the art could easily determine other antimicrobial agents useful in combination with polyvalent bacteriophages.

Pathogen	Antimicrobial or antimicrobial group
E. coli	
Uncomplicated urinary tract infection	trimethoprim-sulfamethoxazole (abbrev. TMO-SMO), or ampicillin; 1st generation cephalosporins, ciprofloxacin
Systemic infection	ampicillin, or a 3rd generation cephalosporin; aminoglycosides, aztreonam, or a penicillin + a pencillinase inhibitor
Klebsiella pneumoniae	1st generation cephalosporins; 3rd gener. cephalosporins, cefotaxime, moxalactam, amikacin, chloramphenicol
Shigella (various)	ciprofloxacin; TMO-SMO, ampicillin, chloramphenicol
Salmonella:	
s. typhi	chloramphenicol; ampicillin or TMO-SMO
Non-typhi species	ampicillin; chloramphenicol, TMO-SMO, ciprofloxacin
Yersinia pestis	streptomycin; tetracycline, chloramphenicol
Enterobacter cloacoe	3rd generation cephalosporins, gentamicin, or tobramycin; carbenicillin, amikacin, ztreonam, imipenem
Hemophilus influenzae:	
Meningitis	chloramphenicol or 3rd generation cephalosporins; ampicillin
Other *H. infl.* Infections	ampicillin; TMO-SMO, cefaclor, cefuroxime, ciprofloxacin
Mycobacterium tuberculosis and M. avium-intracellulare	isoniazid (INH) + rifampin or rifabutin, the above given along with pyrazinamide +/or ethambutol

Neisseria:

N. meningitidis	penicillin G; chloramphenicol, or a sulfonamide

N. gonorrhoeae:

Penicillin-sensitive	penicillin G; spectinomycin, ceftriaxone
Penicilin-resistant	ceftriaxone; spectinomycin, cefuroxime or cefoxitin, ciprofloxacin
Pseudomonas aeruginosa	tobramycin or gentamycin (+/- carbenicillin, aminoglycosides); amikacin, ceftazidime, aztreonam, imipenem

Staph aureus

non-penicillinase producing	penicillin G; 1st generation cephalosporins, vancomycin, imipenem, erythromycin
Penicillinase producing	a penicillinase-resisting penicillin; 1st generation cephalosporins, vanco-mycin, imipenem, erythromycin
Streptococcus pneumoniae	penicillin G; 1st generation cephalosporins, erythromycin, chloramphenicol
Vibrio cholera	tetracycline; TMO-SMO

In another embodiment of the present invention, the polyvalent bacteria of the invention are provided as compositions useful in the treatment and prevention of various bacterial infections, such as diarrhea, dysentery, hemolytic uremic syndrome, bladder infection, kidney infection, urinary tract infection, septicemia, pneumonia, and meningitis, and other various diseases, syndromes, and disorders.

The polyvalent bacteriophage of the invention can be used for the treatment or prevention of _Hemophilus influenza_, _Pseudomonas_, _Streptococcus pneumoniae_, _Streptococcus fasciae_, _Streptococcus_ group B, _Listeria_, _Salmonella_, _E. coli_, _Campylobacter_, and other bacteria, and any combination thereof. For example, if there is a bacterial infection of the upper respiratory tract, the infection can be prophylactically or therapeutically treated with a composition comprising at least one

polyvalent bacteriophage specific for that bacteria, and a carrier for delivering the polyvalent bacteriophage to a mouth, throat, or nasal passage. If an individual has been exposed to someone with the upper respiratory disorder, the polyvalent bacteriophage will reside in the mucosal lining and prevent any colonization of the infecting bacteria.

5   Two examples of bacteria which infect the upper respiratory system are *Streptococcus pneumoniae* and *Hemophilus influenzae*. In recent years, there has been an increase in the number of people, particularly children and the elderly, that are infected or are carriers of penicillin resistant *Streptococcus pneumoniae* and *Hemophilus*. While these bacteria are normally harmless residents of the host, they are
10   opportunistic organisms that are able to cause infections when the resistance of the host has been compromised. By eliminating or reducing the number of these organisms in the upper respiratory tract, there will be a commensurate reduction in the number of infections by these bacteria.

The *Hemophilus* bacteria is infected by bacteriophage HP1 (a member of the P2-
15   like phage family with strong similarities to coliphages P2 and 186, and some similarity to the retronphage Ec67), which produces a lytic enzyme capable of lysing the bacteria. *Streptococcus pneumoniae* is infected with the Pal bacteriophage, which produces a lytic enzyme identified as an N-acetyl-muramoyl-L-alanine amidase. The pharmaceutical composition of the invention can contain either one polyvalent
20   bacteriophage that recognizes these two bacteria, and may contain other polyvalent bacteriophage for other bacteria. The composition which may be used for the prophylactic and therapeutic treatment of a strep infection includes the polyvalent bacteriophage and a means of application, (such as a carrier system or an oral delivery mode), to the mucosal lining of the oral and nasal cavity, such that the polyvalent
25   bacteriophage is put in the carrier system or oral delivery mode to reach the mucosal lining. Another infection which can be treated prophylactically is *Streptococcus* group A, which can produce what is commonly known as "strep" throat. Group A *Streptococci* are infected with a C1 bacteriophage, which produces a lysing enzyme specific for the lysing of *Streptococcus* group A.

30   Another use of a polyvalent bacteriophage of the invention is for the treatment of bacterial infections of the digestive tract. The method for treating a bacterial infection

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of the digestive tract comprises treating the bacterial infection with composition comprising an effective amount of at least one polyvalent bacteriophage specific for the bacteria, and a carrier for delivering said polyvalent bacteriophage to the digestive tract. In a preferred embodiment of the invention, the bacterial infections being treated are

5   being caused by gram negative bacteria selected from the group consisting of *Listeria*, *Salmonella*, *E. coli*, and *Campylobacter*. However, this method and composition will effectively treat other bacteria, when the appropriate polyvalent bacteriophage is used.

Another composition and use of the polyvalent bacteriophage of the invention is for the therapeutic or prophylactic treatment of bacterial infections of burns and wounds

10   of the skin. The composition comprises an effective amount of at least one polyvalent bacteriophage specific for the bacteria and a carrier for delivering at least one polyvalent bacteriophage to the wounded skin. The polyvalent bacteriophage may be applied to a bandage either directly or in one or another carrier. The bandages may be sold damp or dry, wherein the polyvalent bacteriophage is in a lyophilized form on the bandage. This

15   method of application is most effective for the treatment of burns. In some embodiments of the invention, polyvalent bacteriophage for *Pseudomonas*, *Staphylococcus*, and *Streptococcus*, jointly or individually, may be incorporated into one or another carrier, or into a bandage to be used on burn patients.

Yet another use of polyvalent bacteriophages is for the bacterial infections

20   caused by K1 and/or K5 strains of *E. coli*. These bacteria are involved in a variety of infections, the most common are urinary tract infections (UTI). The polyvalent bacteriophage of the present invention would be applied directly to the site of infection, in the case of UTI this would mean to deliver the phage to the bladder through a catheter.

25   In the case of septicemias caused by K1 *E coli*, the polyvalent phage of the present invention could be injected directly into the circulatory system or intraperitoneally.

In case of meningitis caused by *E. coli*, the polyvalent phage of the present invention will be delivered to the cerebro-spinal fluid or directly applied to the brain or

30   meninges.

Yet another use of the polyvalent bacteriophages of the invention is for the prophylactic or therapeutic treatment of vaginal infections. This treatment comprises treating the vaginal infection with an effective amount of at least one polyvalent bacteriophage specific for that bacteria, wherein that polyvalent bacteriophage is

5   incorporated in a carrier to be placed in a vagina. The preferred carrier is a tampon, or vaginal douche. A pad may also be used as a carrier, although it is not as effective. While any number of bacteria could be treated using this composition and method, it is believed that the most optimum use of this treatment composition and method would be for the treatment of an *E. coli* and *Streptococcus* B infection. Vaginal infections caused

10 by Group B *Streptococcus* can cause neonatal meningitis resulting in brain damage and premature death. Polyvalent bacteriophage incorporated into tampons specific for group B Strep would eliminate the group B organisms without disturbing normal flora so that women would not be overcome by yeast infections post antibiotic therapy. The use of the polyvalent bacteriophage in the vagina would best provide a prophylactic effect,

15 although therapeutic use would also be advisable.

Another use of the invention is for the prophylactic and therapeutic treatment of eye infections. The method of treatment comprises administering eye drops which comprise an effective amount of at least one polyvalent bacteriophage specific for the bacteria and a carrier capable of being safely applied to an eye, with the carrier

20 containing the polyvalent bacteriophage. In some embodiments of the invention, the bacteria being treated is *Hemophilus* or *Staphylococcus*. The eye drops are in the form of an isotonic solution.

Polyvalent bacteriophage can also be used to fight dental caries. Specifically, a polyvalent bacteriophage specific for *Streptococcus mutans* may be incorporated in a

25 toothpaste or oral wash. Similarly, this polyvalent bacteriophage may also be incorporated into a chewing gum or lozenge. Any other carrier can be used that allows for the exposure of the mouth, gums, and teeth to the polyvalent bacteriophage.

The routes of administration include but are not limited to oral, aerosol, intranasal, intravenous, intramuscular, intraperitoneal, intrathecal, vaginal, rectal,

30 topical, lumbar puncture, and direct application to the brain or meninges. Pharmaceutically acceptable excipients which can be used as a vehicle for the delivery

of the phage will be apparent to those skilled in the art. For example, the free phage could be in lyophilized form and be dissolved just prior to administration by IV injection. The dosage of administration is contemplated to be in the range of about 10^6 to about 10^{13} pfu/per kg/per day, and preferably about 10^{12} pfu/per kg/per day. The

5   phage are administered until successful elimination of the pathogenic bacteria is achieved.

With respect to the aerosol administration to the lungs, the polyvalent bacteriophage is incorporated into an aerosol formulation specifically designed for administration to the lungs by inhalation. Many such aerosols are known in the art, and

10   the present invention is not limited to any particular formulation. An example of such an aerosol is the Proventil™ inhaler manufactured by Schering-Plough, the propellant of which contains trichloromonofluoromethane, dichlorodifluoromethane and oleic acid. The concentrations of the propellant ingredients and emulsifiers are adjusted if necessary based on the phage being used in the treatment. The number of phage to be

15   administered per aerosol treatment will be in the range of 10^6 to 10^{13} pfu, and preferably 10^{12} pfu.

Isolation and Characterization of ΦK1-5

ΦK1-5 was isolated using *E. coli* ATCC 23506 (K5) as a host. Electron micrographs show that ΦK1-5 is morphologically similar to the *Podoviridae* family,

20   which includes coliphages T7, T3, and *Salmonella* phages SP6 and P22. The phage particle consists of an icosohedral head of about 60 nm in diameter with a small tuft of short tail fibers (Figure 1). ΦK1-5 is highly lytic. When phage were added to a logarithmic culture of a susceptible host at an multiplicity of infection (moi) of 1:1, lysis occurs in 15-20 min. Burst size was determined by a one step growth curve and found to

25   be to be about 110. ΦK1-5 plaques are clear and large, about 4.0-5.0 mm in diameter with a halo of about 12.0-15.0 mm in diameter on LB agar plates. The plaques reached a limit in size after 24 hours. In contrast, T7 plaques, can continue to grow for several days (38). DNA was isolated from cesium chloride density gradient purified phage by phenol extraction. Digestion of the DNA with several restriction enzymes indicated that

30   it is double stranded, with an estimated size of 40 kb.

Extended host range of ΦK1-5

The host range of ΦK1-5 was compared to that of ΦK1E (K1 antigen specific) and ΦK5 (K5 antigen specific). *E. coli* strains ATCC 23506 and ATCC 23508 possess the K5 polysaccharide capsule, and strains ATCC 23503 and ATCC 23511 possess the K1 capsule. Also tested was a set of K5 strains collected by Ian Roberts from the University of Manchester and a set of K1 isolates collected by Richard Silver from the University of Rochester (Table 1). ΦK1-5 is able to infect and grow on all of the K1 and K5 strains, ΦK1E only grows on the K1 strains and ΦK5 only grows on the K5 strains. ΦK1E, ΦK5, and ΦK1-5 were also tested for growth on the following ATCC strains: 23502 (K4), 23504 (K8), 23505 (K9), 23507 (K10), 23509 (K11), 23510 (K14), 23515 (K17), 23516 (K20), 23517 (K13), 23518 (K18), 19110 (K7), 19138 (K2) and 31616 (K35). No phage growth by ΦK1-5, ΦK1E or ΦK5 was seen on any of these strains.

TABLE 1. Host ranges of phages ΦK1E, ΦK5, ΦK1–5, ΦK1–5$_{(K1^-)}$, and ΦK1–5$_{(K5^-)}$[a]

E. coli strain	K antigen	ΦK1-5	ΦK1E	ΦK5	ΦK1-5$_{(K1^-)}$	ΦK1-5$_{(K5^-)}$
ATTCC 23503	K1	+	+	-	-	+
ATCC 23511	K1	+	+	-	-	+
RS164	K1	+	+	-	-	+
RS166	K1	+	+	-	-	+
RS167	K1	+	+	-	-	+
RS168	K1	+	+	-	-	+
RS176	K1	+	+	-	-	+
RS179	K1	+	+	-	-	+
RS180	K1	+	+	-	-	+
RS188	K1	+	+	-	-	+
RS203	K1	+	+	-	-	+
RS215	K1	+	+	-	-	+
RS218	K1	+	+	-	-	+
RS228	K1	+	+	-	-	+
ATCC 23506	K5	+	-	+	+	-
ATCC 23508	K5	+	-	+	+	-
20026	K5	+	-	+	+	-
21195	K5	+	-	+	+	-
21386	K5	+	-	+	+	-
21786	K5	+	-	+	+	-
21795	K5	+	-	+	+	-
21831	K5	+	-	+	+	-
21832	K5	+	-	+	+	-
21834	K5	+	-	+	+	-
21835	K5	+	-	+	+	-

5   [a]Plaque assays were done to determine host range of the phages against a collection of K1 and K5 strains of E. coli. ΦK1E only grows on K1 strains, ΦK5 only grows on ΦK5 strains and ΦK1-5 grows on both. ΦK1-5$_{(K1^-)}$ and ΦK1-5$_{(K5^-)}$ are mutants of ΦK1-5 defective in growth on K1 and K5 strains, respectively.

10   Because of the promoter sequence similarity between ΦK1-5 and SP6, we tested if ΦK1-5 could grow on Salmonella typhimurium strain LT2 (the host for SP6) and if SP6 could grow on any of the E. coli isolates sensitive to ΦK1-5. SP6 did not grow on any of the E. coli strains, and likewise ΦK1-5 did not grow on Salmonella typhymurium.

15

ΦK1-5 encodes two tail genes

ΦK1E and ΦK5 share a region of sequence similarity upstream of the tail proteins (including the SP6-like promoter, 3). Since ΦK1-5 had structural, biological and host similarities to these two phages, we speculated that all three may be closely related to and share this upstream sequence similarity. We designed a primer based on the sequence of this region in ΦK1E and ΦK5 to determine the sequence downstream of the promoter. When ΦK1-5 DNA was used as a template, the primer did hybridize, and we were able to generate sequence. We continued sequencing downstream by primer walking. The sequence immediately downstream of the promoter was very similar to ΦK5, and encoded an open reading frame with a high degree of sequence similarity (>92% amino acid identity) to that of ΦK5 tail protein. Continued sequencing downstream revealed a second open reading frame that is nearly identical (>97% amino acid identity) to the endosialidase protein of ΦK1E. An intergenic region of 85 base pairs lies between the termination codon of the lyase gene and the start codon of the endosialidase gene. This region is also present in ΦK5, immediately following the K5 lyase gene, and also in ΦK1E, immediately upstream of the endosialidase gene and immediately downstream of a 111 amino acid open reading frame (ORF$_L$, 6). No recognizable promoter was found in this region, but there are two strong regions of symmetry, which may act as a Rho-independent transcriptional terminator. Sequence was determined 598 base pairs downstream of the termination codon of the endosialidase gene, at which point the end of the DNA molecule was reached. No open reading frames were found in this area.

The sequence 500 base pairs upstream of the K5 lyase gene in ΦK1-5 was also determined. Like in the other phages, an SP6-like promoter is present and is probably required for transcription of the tail genes. The upstream sequence shares a high degree (>90%) of identity to that of the analogous region in ΦK5 and ΦK1E.

We also sequenced downstream of the endosialidase gene of ΦK1E; 718 base pairs downstream from the endosialidase termination codon we reached the end of the DNA molecule. There is little sequence similarity between this region and the analogous region in ΦK1-5.

Each ΦK1-5 virion contains both tail proteins

We addressed the question of whether ΦK1-5 particles contain both tail fiber proteins, or if two populations of particles (one containing the K5 lyase and the other containing the endosialidase) were produced after infection. We made a phage preparation using ATCC 23506 (K5) as a host and determined its titer on ATCC 23506 (K5) and ATCC 23503 (K1) (Table 2). A sample of the phage was then incubated with ATCC 23506 for 5 min, which is long enough for phage to attach and possibly inject the DNA but not long enough for production of new phage particles. The MOI was 1/100 phage particle/bacteria. The mixture was then rapidly filtered. Phage particles that had attached to the cells would be eliminated from the filtrate. The filtrate was then titered on both the K1 and K5 strains. If the phage preparation was initially a mixture of two populations, then only those displaying the K5 lyase would attach and be eliminated. The remaining phage would be mainly those that contained the K1 specific endosialidase, and therefore the titer would be higher on the K1 *E. coli* strains than on the K5 strain. On the other hand, if each of the phage particles contained both tail proteins, titers of the phage remaining in the filtrate would be the same on the two strains, i.e., levels of the K5 lyase containing phages would not be selectively reduced. We found the latter to be the case and concluded that each virion has both the K1 endosialidase and the K5 lyase. Similar results were seen in the converse experiment in which the 5-min incubation was performed using the K1 *E. coli* strain (Table 2). As controls we performed the experiments with both ΦK1E and ΦK5, using both strains for the incubation. ΦK1E titers were reduced 99% by pre-incubation with the K1 strain but not with the K5 strain, and ΦK5 titers were similarly reduced after pre-incubation with the K5 strain but not with the K1 strain.

TABLE 2. Preincubation experiments to show that all ΦK1-5 particles contain both tail proteins[a]

Phage	Titer
ΦK1-5	
Preincubated with 23506	5.2×10^8
Titered on 23506 after preincubation	3.1×10^6
Titered on 23503 after preincubation	3.7×10^6
Preincubated with 23503	5.2×10^8
Titered on 23506 after preincubation	6.8×10^6
Titered on 23503 after preincubation	6.4×10^6
ΦK5	
Preincubated with 23506	4.0×10^8
Titered on 23506 after preincubation	8.5×10^5
Preincubated with 23503	4.0×10^8
Titered on 23506 after preincubation	3.3×10^8
ΦK1E	
Preincubated with 23506	7.7×10^8
Titered on 23503 after preincubation	6.8×10^8
Preincubation with 23503	7.7×10^8
Titered on 23503 after preincubation	3.0×10^6

5

[a]Preincubation of ΦK1-5 with either ATCC 23503 (K1) or ATCC 23506 (K5) for 5 min results in roughly 100-fold loss of phage particles when titered on either strain. If the phage preparation consisted of two populations that each contained just one tail protein, then titers after the incubation would be reduced only on the strain that was 10 used in the preincubation. ΦK5 titers are reduced by preincubation with a permissive host (23506) but not with a nonpermissive host (23503). ΦK1E titers are also reduced only by preincubation on a permissive host (23503) and not a nonpermissive host (23506).

15   <u>ΦK1-5 mutants defective in growth on either K1 or K5 *E. coli*</u>

A mixed lawn of K1 and K5 strains of *E. coli* was used to screen for ΦK1-5 mutants defective in growth on one or the other host strains. ΦK1-5 forms clear plaques on a mixed lawn of K1 and K5 *E. coli*; mutants in either tail would result in turbid plaques due to growth of the non-permissive host. Phage were treated with the mutagen

hydroxylamine and plated on a double lawn. Turbid plaques were identified, picked, and purified by multiple plaque isolations on the double lawn. These were then screened by separately testing for growth on each strain. Of eight isolates purified, three were unable to plaque on the K5 strain but could plaque on the K1 strain. One of these, ΦK1-5$_{(K5-)}$, was screened for growth against the entire host collection and found to be unable to replicate on any of the K5 strains but was able to grow on all of the K1 strains (Table 1). Five of the isolates could still replicate on both K1 and K5 strains but gave a turbid plaque morphology on the on the K5 strains.

None of the mutants isolated in this way were defective in growth on K1 strains, so we devised a selection/amplification scheme to enrich for those that can replicate on K5 but not K1 hosts. Mutagenized phage were amplified on a K5 strain, filtered to remove bacterial debris, then used to infect a logarithmically growing K1 strain for 5 minutes. This mixture was rapidly filtered before phage burst could occur. Phage able to grow on K1 strain would attach to the cells and be eliminated from the filtrate. The sample was then re-amplified on the K5 strain and the cycle was repeated eight times. This strongly selects for phage that can replicate on K5 hosts but not K1 hosts. Titers of the filtrate were 200 fold higher on the K5 strain than on the K1 strain. Several were picked and purified by multiple rounds of single plaque isolation. One isolate, ΦK1-5$_{(K1-)}$, was further characterized and found to be unable to grow on any of the K1 strains (Table 1).

DNA sequence of a putative ΦK5 tail gene

Clarke et. al. described a partial sequence of an open reading frame (ORFp) in ΦK5 immediately downstream of the 85 base region common to the three phages (6). We continued sequencing downstream and found the complete open reading frame is 523 amino acids. A BLAST search revealed a small region of sequence similarity with the N-acetylglucosamine-permease IIABC component near the N-terminus. It has no significant sequence similarity with any other entry in the database or any of the tail proteins described here. Sequence was determined an additional 163 bases downstream, at which point the end of the DNA molecule was reached.

Figure 2 compares the regions encoding tail proteins in all three phages. ΦK1-5 has a K5 lyase protein in the same position as that of ΦK5. ΦK1E has a 111 amino acid open reading frame (ORF$_L$) of unknown function in this position. Immediately downstream all three phages have an intergenic region of 85 bases that has two dyad

5   axis of symmetry. Immediately downstream of this region ΦK1-5 encodes its endosialidase protein, which is in the analogous position as the ΦK1E endosialidase. ΦK5 encodes a 523 amino acid open reading frame (ORF$_P$) in this position. The three phages share sequence similarity upstream of the tail genes. No sequence similarity was noted downstream, and in all three phages the DNA molecule ends downstream.

10   EXAMPLE 1

Isolation of K1-5

ΦK1-5 was isolated from raw sewage using the plaque technique. Briefly, a 1L sample of sewage was centrifuged at 6000 rpm in a GSA rotor to remove solid matter and was then passed through a 0.45 micron nitrocellulose filter (Nalgene). 100 μl of

15   filtrate was added to 200 μl of an overnight culture of *E. coli* ATCC 23506 (K5) grown in LB media. 3 ml of melted tempered top agar (5 g/L agar in LB) was added and the mix was plated onto an LB agar plate and incubated at 37°C overnight. The following day plaques were picked and re-plaqued 3 times to insure pure culture. Final plaque isolates were stored as an agar plug from a Pasteur pipette deposited in 1 ml of SM

20   buffer (10 mM MgSO$_4$, 100 mM NaCl, 0.01% gelatin, 50 mM Tris pH 7.5).

Host range was initially screened by spotting 10 μl of SM buffer containing a plaque plug onto a lawn of an appropriate strain. Host range of interesting phage isolates was further confirmed by the plaque assay. All phage titrations were done by the plaque assay technique.

25   **Large Scale Purification**

Phages were prepared by the cesium chloride density gradient method. 1 L of an appropriate host was grown up to and OD 600 of between 0.4 and 0.6 at 37°C with 200 rpm shaking in LB broth. Phage were added at a moi of 1 phage/100 bacteria, and the culture was allowed to incubate until the OD reached a minimum for 30 min. 10 ml of

30   chloroform was added and allowed to shake for 10 min and was then centrifuged for 20

min at 6000 rpm in GSA rotor to remove cellular debris. The supernatant was collected and 1/10th volume of 5M NaCl and 1/10th w/v of polyethylene glycol was added to precipitate the phage, this was held at 4°C overnight. The phage were then pelleted by centrifugation at 6000 rpm in a GSA rotor at 4°C. The pellet was resuspended in phosphate buffered saline and CsCl was added to a density of 1.5 g/ml. The sample was spun in Ti80 (Beckman) rotor at 34,000 rpm overnight. The phage band was extracted with a syringe and was dialyzed against phosphate buffered saline (pH 7.4).

DNA Isolation and Sequencing

DNA was isolated from CsCl purified phage by phenol/chloroform extraction. The phage DNA was used directly as a template for DNA sequencing which was carried out by Commonwealth Biotechnologies in Richmond, VA . Both strands were sequenced (of the tail gene region of θK1-5). DNA database searches were done by BLAST (1), and sequence alignments were performed with the Wisconsin Package (9).

Mutagenesis

Cesium purified phage were mutagenized with UV light using a model TM 36 chromatovue transilluminator (UVP, Inc.). Phage were typically exposed for 10-20 sec, which reduced viability by 1000 fold. The mutagenized phage were then amplified on ATCC 23503 or ATCC 23506 and subjected to selection and amplification as described above. Phage were also mutagenized by incubation with 400 mM hydroxylamine until the phage titer was reduced by 100 fold. They were then plated on a double lawn of ATCC 23503 and ATCC 23506. Turbid plaques were picked, replaqued for isolation, and tested for growth against a collection of K1 and K5 E. coli strains.

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While the present invention has been described in some detail for purposes of clarity and understanding, one skilled in the art will appreciate that various changes in form and detail can be made without departing from the true scope of the invention. All figures, tables, and appendices, as well as patents, applications, and publications, referred to above, are hereby incorporated by reference.

WHAT IS CLAIMED IS:

1. A pharmaceutical composition comprising a phage having multiple host specificity based on having multiple different host tail proteins and a pharmaceutically acceptable excipient.

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2. A pharmaceutical composition according to claim 1, said phage having multiple different hydrolytic tail proteins.

3. A pharmaceutical composition according to claim 1, said phage having
10   multiple different K specific hydrolytic tail proteins.

4. A pharmaceutical composition according to claim 1, said phage comprising the full length genome of ΦK1-5 on deposit as ATCC Accession No. PTA-3495 irrespective of the tail gene region.

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5. A pharmaceutical composition according to claim 1, said phage comprising the full length genome of ΦK1-5 on deposit as ATCC Accession No. PTA-3495 including the tail gene region.

20   6. A pharmaceutical composition according to claim 1, said phage comprising the full length genome of ΦK1E.

7. A pharmaceutical composition according to claim 1, said phage comprising the full length genome of ΦK5.

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8. A pharmaceutical composition according to claim 1, said phage comprising the full length genome of ΦK20.

9. A pharmaceutical composition according to claim 1, wherein said phage
30   infects *Escherichia* and additionally infects a bacterium selected from the group consisting of *Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Enterococcus, Clostridium, Corynebacterium,*

Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia and *Klebsiella.*

10. A pharmaceutical composition according to claim 1, wherein said phage infects *Shigella* and additionally infects a bacterium selected from the group consisting of *Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Enterococcus, Clostridium, Corynebacterium, Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia* and *Klebsiella.*

11. A pharmaceutical composition according to claim 1, wherein said phage infects *Salmonella* and additionally infects a bacterium selected from the group consisting of *Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Enterococcus, Clostridium, Corynebacterium, Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia* and *Klebsiella.*

12. A pharmaceutical composition according to claim 1, wherein said phage infects *Enterobacter* and additionally infects a bacterium selected from the group consisting of *Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Enterococcus, Clostridium, Corynebacterium, Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia* and *Klebsiella.*

13. A pharmaceutical composition according to claim 1, wherein said phage infects *Yersinia* and additionally infects a bacterium selected from the group consisting of *Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Enterococcus, Clostridium, Corynebacterium, Mycobacterium,*

Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia and *Klebsiella.*

14. A pharmaceutical composition according to claim 1, wherein said phage infects *Vibrio* and additionally infects a bacterium selected from the group consisting of *Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Enterococcus, Clostridium, Corynebacterium, Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia* and *Klebsiella.*

15. A pharmaceutical composition according to claim 1, wherein said phage infects *Legionella* and additionally infects a bacterium selected from the group consisting of *Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Enterococcus, Clostridium, Corynebacterium, Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia* and *Klebsiella.*

16. A pharmaceutical composition according to claim 1, wherein said phage infects *Pseudomonas* and additionally infects a bacterium selected from the group consisting of *Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Enterococcus, Clostridium, Corynebacterium, Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia* and *Klebsiella.*

17. A pharmaceutical composition according to claim 1, wherein said phage infects *Neisseria* and additionally infects a bacterium selected from the group consisting of *Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Enterococcus, Clostridium, Corynebacterium, Mycobacterium,*

Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia and *Klebsiella.*

18. A pharmaceutical composition according to claim 1, wherein said phage infects *Bordetella* and additionally infects a bacterium selected from the group consisting of *Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Enterococcus, Clostridium, Corynebacterium, Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia* and *Klebsiella.*

19. A pharmaceutical composition according to claim 1, wherein said phage infects *Helicobacter* and additionally infects a bacterium selected from the group consisting of *Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Enterococcus, Clostridium, Corynebacterium, Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia* and *Klebsiella.*

20. A pharmaceutical composition according to claim 1, wherein said phage infects *Listeria* and additionally infects a bacterium selected from the group consisting of *Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Enterococcus, Clostridium, Corynebacterium, Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia* and *Klebsiella.*

21. A pharmaceutical composition according to claim 1, wherein said phage infects *Staphylococcus* and additionally infects a bacterium selected from the group consisting of *Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Enterococcus, Clostridium, Corynebacterium,*

Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia and *Klebsiella.*

22. A pharmaceutical composition according to claim 1, wherein said phage
infects *Streptococcus* and additionally infects a bacterium selected from the group
consisting of *Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio,
Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria,
Staphylococcus, Streptococcus, Enterococcus, Clostridium, Corynebacterium,
Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus,
Serratia* and *Klebsiella.*

23. A pharmaceutical composition according to claim 1, wherein said phage
infects *Enterococcus* and additionally infects a bacterium selected from the group
consisting of *Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio,
Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria,
Staphylococcus, Streptococcus, Enterococcus, Clostridium, Corynebacterium,
Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus,
Serratia* and *Klebsiella.*

24. A pharmaceutical composition according to claim 1, wherein said phage
infects *Clostridium* and additionally infects a bacterium selected from the group
consisting of *Escherichia, Shigella, Salmonella. Enterobacter, Yersinia, Vibrio,
Legionella, Pseudomonas, Neisseria, Bordetella. Helicobacter, Listeria,
Staphylococcus, Streptococcus, Enterococcus. Clostridium, Corynebacterium,
Mycobacterium, Treponema, Borrelia, Campylobacter. Chlamydia, Haemophilus,
Serratia* and *Klebsiella.*

25. A pharmaceutical composition according to claim 1, wherein said phage
infects *Corynebacterium* and additionally infects a bacterium selected from the group
consisting of *Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio,
Legionella, Pseudomonas, Neisseria, Bordetella. Helicobacter, Listeria,
Staphylococcus, Streptococcus, Enterococcus, Clostridium, Corynebacterium,*

Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia and *Klebsiella.*

26. A pharmaceutical composition according to claim 1, wherein said phage

5   infects *Mycobacterium* and additionally infects a bacterium selected from the group consisting of *Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Enterococcus, Clostridium, Corynebacterium, Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus,*

10   *Serratia* and *Klebsiella.*

27. A pharmaceutical composition according to claim 1, wherein said phage infects *Treponema* and additionally infects a bacterium selected from the group consisting of *Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio,*

15   *Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Enterococcus, Clostridium, Corynebacterium, Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia* and *Klebsiella.*

20   28. A pharmaceutical composition according to claim 1, wherein said phage infects *Borrelia* and additionally infects a bacterium selected from the group consisting of *Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Enterococcus, Clostridium, Corynebacterium, Mycobacterium,*

25   *Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia* and *Klebsiella.*

29. A pharmaceutical composition according to claim 1, wherein said phage infects *Campylobacter* and additionally infects a bacterium selected from the group

30   consisting of *Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Enterococcus, Clostridium, Corynebacterium,*

Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia and *Klebsiella.*

30. A pharmaceutical composition according to claim 1, wherein said phage infects *Chlamydia* and additionally infects a bacterium selected from the group consisting of *Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Enterococcus, Clostridium, Corynebacterium, Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia* and *Klebsiella.*

31. A pharmaceutical composition according to claim 1, wherein said phage infects *Haemophilus* and additionally infects a bacterium selected from the group consisting of *Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Enterococcus, Clostridium, Corynebacterium, Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia* and *Klebsiella.*

32. A pharmaceutical composition according to claim 1, wherein said phage infects *Serratia* and additionally infects a bacterium selected from the group consisting of *Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Enterococcus, Clostridium, Corynebacterium, Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia* and *Klebsiella.*

33. A pharmaceutical composition according to claim 1, wherein said phage infects *Klebsiella* and additionally infects a bacterium selected from the group consisting of *Escherichia, Shigella, Salmonella, Enterobacter, Yersinia, Vibrio, Legionella, Pseudomonas, Neisseria, Bordetella, Helicobacter, Listeria, Staphylococcus, Streptococcus, Enterococcus, Clostridium, Corynebacterium,*

Mycobacterium, Treponema, Borrelia, Campylobacter, Chlamydia, Haemophilus, Serratia and *Klebsiella*.

34. A pharmaceutical composition according to claim 1, said phage further having a gene encoding a factor that permits said phage to replicate and function in another type of bacteria generally not infected by said phage.

35. A pharmaceutical composition comprising a phage having mammalian host specificity by presenting on the tail of said phage a mammalian cell surface-receptor ligand, wherein the phage genome encodes a therapeutic gene product.

36. A pharmaceutical composition according to the preceding claim, wherein said therapeutic gene product is a protein cytocide, an antisense, a ribozyme, a dominant negative mutant, or a therapeutic protein.

37. A method of making a pharmaceutical composition comprising:

(a) isolating a phage according to claim 1 wherein the acquisition of new tail genes occurs by recombination in nature; and

(b) combining said phage with a pharmaceutically acceptable excipient.

38. A method of making a pharmaceutical composition comprising:

(a) generating a phage according to claim 1 wherein the acquisition of new tail genes is generated by technology in the laboratory; and

(b) combining said phage with a pharmaceutically acceptable excipient.

39. A method of preventing or treating bacterial infection comprising administering the pharmaceutical composition of any of claims 1-34 to an individual in need thereof to prevent or treat bacterial infection.

40. Use of the pharmaceutical composition of any of claims 1-34 for the preparation of a medicament for prevention or treatment of bacterial infection.

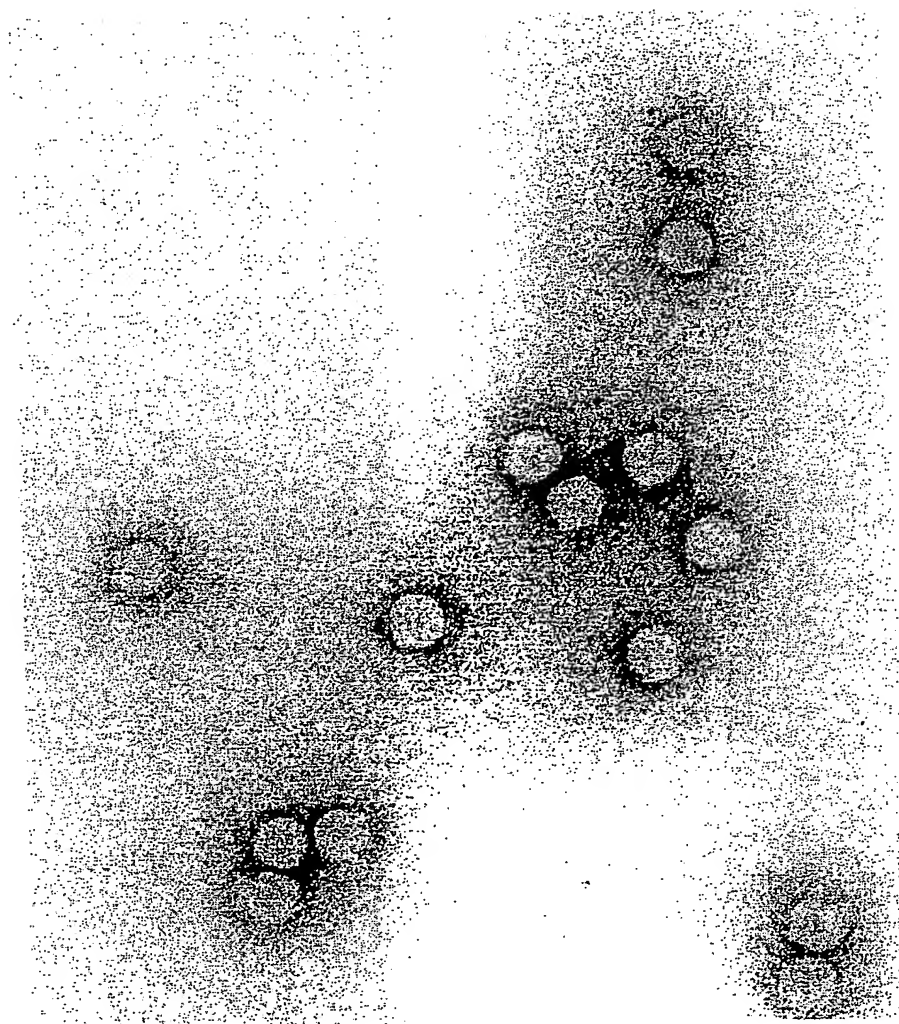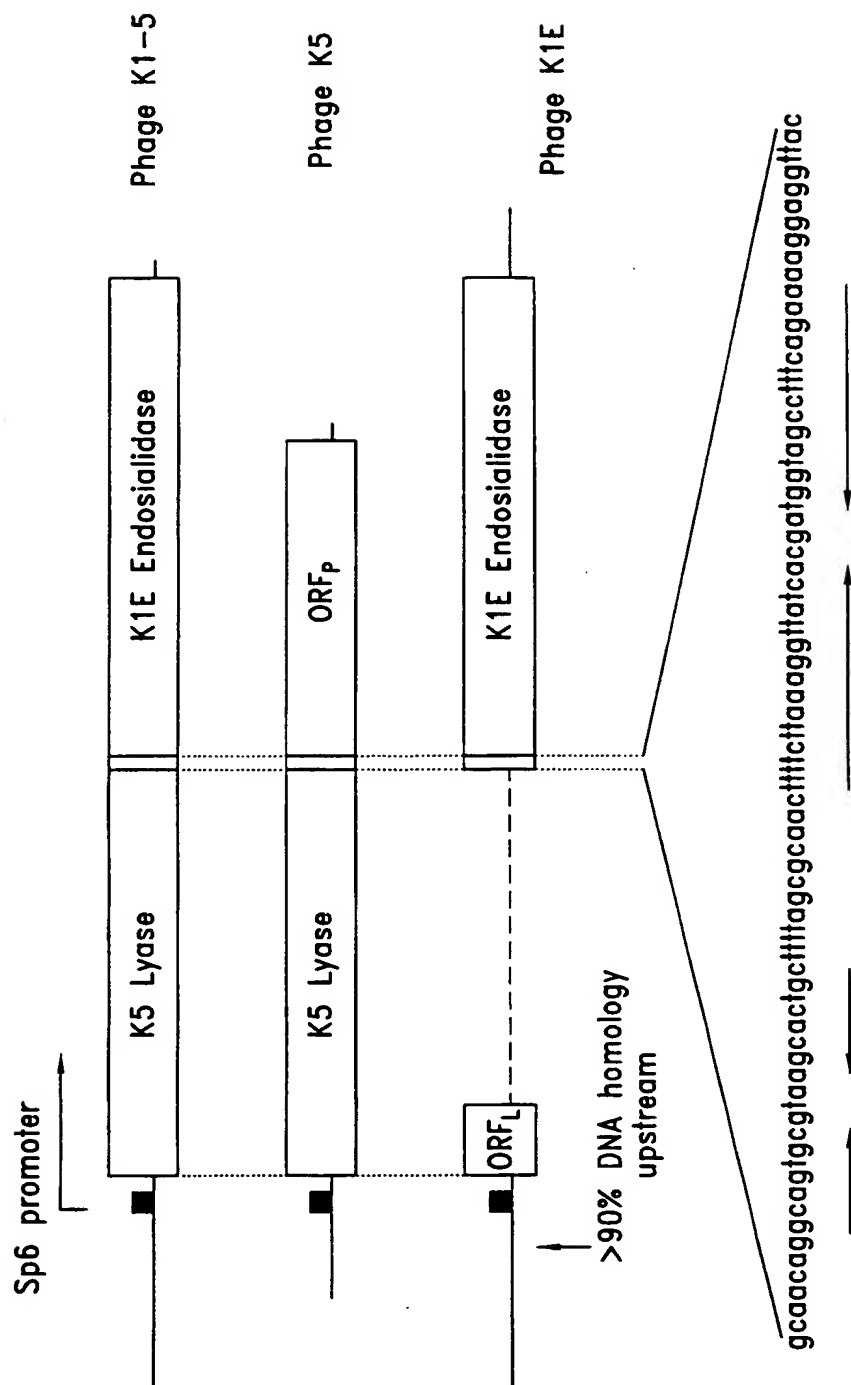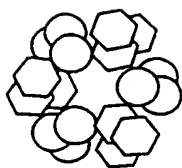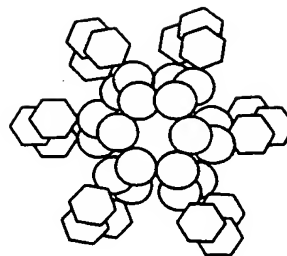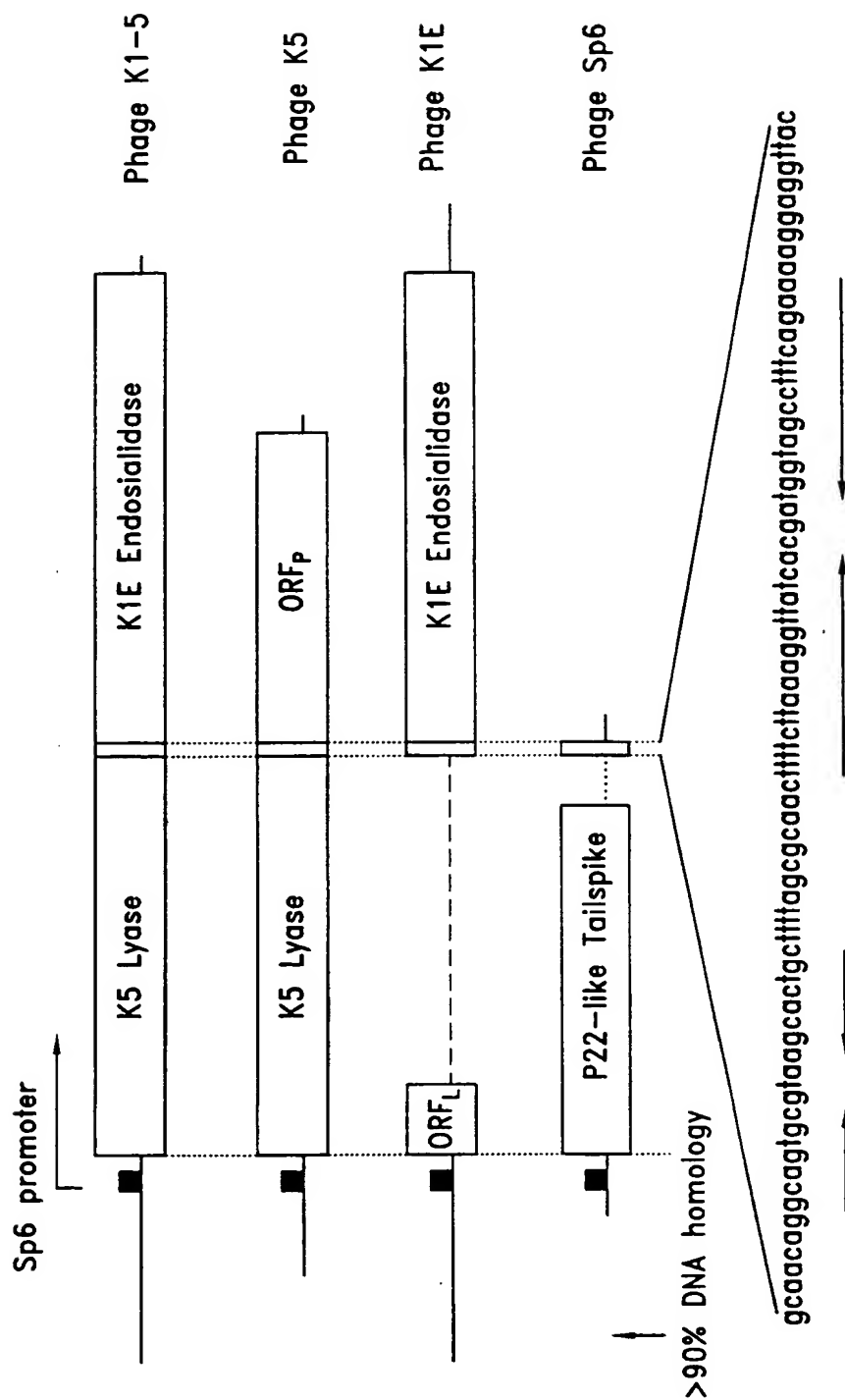FIG. 1

FIG. 2

FIG. 3A

FIG. 3B

FIG. 4

SEQUENCE LISTING

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(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
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— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

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Previous Correction:
see PCT Gazette No. 16/2002 of 18 April 2002, Section II

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: BACTERIOPHAGE HAVING MULTIPLE HOST RANGE

(57) Abstract: The present invention discloses compositions and methods for the prophylaxis and treatment of bacterial infections by the use of polyvalent bacteriophage having multiple host range.

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7   A61K35/76   C12N7/00   C12N15/86   A61P31/04   A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7   A61K   C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

SEQUENCE SEARCH, CHEM ABS Data, SCISEARCH, EMBASE, BIOSIS, MEDLINE, EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CAO J ET AL: "Helicobacter pylori-antigen-binding fragments expressed on the filamentous M13 phage prevent bacterial growth" BBA - GENERAL SUBJECTS, ELSEVIER SCIENCE PUBLISHERS, NL, vol. 1474, no. 1, 6 March 2000 (2000-03-06), pages 107-113, XP004276546 ISSN: 0304-4165 abstract	1-34, 37-40
Y	WO 97 29185 A (MAARDH SVEN) 14 August 1997 (1997-08-14) claim 1	1-34, 37-40

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 May 2002	31/05/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Niemann, F

Form PCT/ISA/210 (second sheet) (July 1992)

page 1 of 3

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	TETART F ET AL: "Bacteriophage T4 host range is expanded by duplications of a small domain of the tail fiber adhesin." JOURNAL OF MOLECULAR BIOLOGY, vol. 258, no. 5, 1996, pages 726-731, XP002199086 ISSN: 0022-2836 cited in the application abstract page 730, left-hand column, paragraph 2 -right-hand column, paragraph 1	1-34, 37-40
Y	TETART F ET AL: "GENOME PLASTICITY IN THE DISTAL TAIL FIBER LOCUS OF THE T-EVEN BACTERIOPHAGE: RECOMBINATION BETWEEN CONSERVED MOTIFS SWAPS ADHESINSPECIFICITY" JOURNAL OF MOLECULAR BIOLOGY, LONDON, GB, vol. 282, no. 3, 1998, pages 543-556, XP000920896 ISSN: 0022-2836 cited in the application abstract page 543, right-hand column, paragraph 2	1-34, 37-40
A	BARROW P A ET AL: "BACTERIOPHAGE THERAPY AND PROPHYLAXIS: REDISCOVERY AND RENEWED ASSESSMENT OF POTENTIAL" TRENDS IN MICROBIOLOGY, ELSEVIER SCIENCE LTD., KIDLINGTON, GB, vol. 5, 1997, pages 268-271, XP000993117 ISSN: 0966-842X cited in the application the whole document	1-34, 37-40
A	MERRIL C R ET AL: "LONG-CIRCULATING BACTERIOPHAGE AS ANTIBACTERIAL AGENTS" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 93, no. 8, 16 April 1996 (1996-04-16), pages 3188-3192, XP002044135 ISSN: 0027-8424 the whole document	1-34, 37-40
P,X	SCHOLL DEAN ET AL: "Bacteriophage K1-5 encodes two different tail fiber proteins, allowing it to infect and replicate on both K1 and K5 strains of Escherichia coli." JOURNAL OF VIROLOGY, vol. 75, no. 6, March 2001 (2001-03), pages 2509-2515, XP002199087 ISSN: 0022-538X the whole document	1-34, 37-40

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INTERNATIONAL SEARCH REPORT

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category°	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 21007 A (VIAGENE INC) 11 July 1996 (1996-07-11) the whole document	35,36
X	WO 98 05344 A (BRIGHAM & WOMENS HOSPITAL ;SARKAR SAUMYENDRA N (US); DUBIN DANIEL) 12 February 1998 (1998-02-12) the whole document	35,36

FURTHER INFORMATION CONTINUED FROM   PCT/ISA/ 210

This International Searching Authority found multiple (groups of)
inventions in this international application, as follows:

1. Claims: 1-34,37-40

    a pharmaceutical composition ·comprising a phage having
    multiple host specificity based on having multiple host tail
    proteins and a pharmaceutically acceptable excipient

2. Claims: 35,36

    a pharmaceutical composition comprising a phage having
    mammalian host specificity by presenting on the tail of said
    phage a mammalian cell surface-receptor ligand, wherein the
    phage genome encodes a therapeutic gene product

INTERNATIONAL SEARCH REPORT

Information on patent family members

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
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